 DOI: https://doi.org/10.5114/ceji.2020.101252

Genetic variants of *SERPING1* gene in Polish patients with hereditary angioedema due to C1 inhibitor deficiency

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Abstract

Hereditary angioedema due to C1-inhibitor deficiency (C1-INH-HAE) type I and II is a rare and life-threatening disease caused by SERPING1 gene mutations. Previous genetic studies indicated a wide spectrum of disease-associated variants in the SERPING1 gene and often lack of correlation with patient's phenotypes. The aim of this study was to evaluate the presence, type, and localization of mutations in the SERPING1 gene in 41 Polish patients with C1-INH-HAE and their relation with case/family history, type of C1-INH-HAE, fC1-INH, age of onset, and disease severity. Sanger sequencing and MLPA method were used for detection of disease-associated variants. In 34 (82.9%) patients, mutations located in various regions of SERPING1 gene were revealed. The detected alterations in patients with C1-INH-HAE type I differed and were positioned in various exons/introns of the SERPING1 gene. The most frequent disease-associated variants appeared in exon 3 (especially in type I) and in exon 8 (type I and II). Out of 20 different disease-causing variants, 9 were not previously described. We did not find any relation between the type and location of the mutations and no type of features included in phenotype evaluation of the patients, such as case and family history, type of C1-INH-HAE, age of onset, biochemical parameters, or severity of disease.

Key words: *hereditary angioedema, C1-inhibitor, SERPING1, disease-associated variants.*

(Cent Eur J Immunol 2020; 45 (3): 301-309)

Introduction

Hereditary angioedema due to C1-inhibitor deficiency (C1-INH-HAE) type I and II is a rare and life-threatening disease caused by *SERPING1* gene mutations inherited as an autosomal dominant trait with incomplete penetrance [1, 2]. After initial recognition in the 80-s, the mutation of *SERPING1* gene encoding C1-inhibitor (*C1-INH*) is responsible for C1-INH-HAE [3, 4], and introducing genetic studies in patients with C1-INH-HAE [5] has become an essential part of molecular researches conducted on this disease [4, 6, 7]. Previous genetic studies indicated a wide spectrum of disease-associated variants in the *SERPING1* gene and often lack of correlation with the phenotype in patients with this disease [8-11]. Over 500 different *SERPING1* mutations [4] in patients with C1-INH-HAE have been detected and described according to the Human Genome Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/index.php).

The aim of our study was to evaluate the presence, type, and localization of the disease-associated variants in the *SERPING1* gene in Polish patients with C1-INH-HAE type I and type II [12] and their relation with patients' phenotype as well as laboratory tests (fC1INH: C1-INH function, aC1INH: antigenic C1-INH) in the context of detected disease-associated variants in the *SERPING1* gene.

Material and methods

The study included 41 patients with C1-INH-HAE registered in Poland (31 females, 10 males, aged 4-75 years old) from 31 families [12]. 34 patients (82.9%) presented with type I, and 7 patients (17.1%) with type II of C1-INH-HAE. Family history was negative for 16 patients. In 19 cases, the course of disease was severe (score 3), in 19 – it was moderate (score 2), in 2 – light (score 1), and 1 patient was asymptomatic (score 0, 4-year-old child with fC1-INH of 76%).

The diagnosis of C1-INH-HAE was based on the case and family history, examination during the angioedema attack, assessment of disease severity with scoring 0-3

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Submitted: 9.07.2019; Accepted: 11.09.2019

Table 1. Primers used for Sanger sequencing analysis of coding region of SERPING1 gene (reference sequence No. ENST00000278407.8)

Exon (exon ref No. in Ensembl)	Primers	Length of PCR product	Annealing temperature in PCR reaction
1. ENSE00001941568	F: 5'-CAC CTA CCA GGG GAT TTG G-3' R: 5'-ACC CCC TCC CTA GAC CTC TT-3'	319 bp	58°C
2. ENSE00002142743	F: 5'-TGG GGA AAA CAA AAC AGA GG-3' R: 5'-CGG AGC CTG AAG GGT TAA T-3'	346 bp	56°C
3. ENSE00003463488	F: 5'-CCA CAC CTT CTC TTC CTG CT-3' R: 5'-CAT GGC TTT GTA AGT GTC TGG A-3'	668 bp	59°C
4. ENSE00003528440	F: 5'-CTC CAT TCC AGC CTG GTC-3' R: 5'-GCT CCA ACA TTC CCT CTG TC-3'	328 bp	58°C
5. ENSE00003567744	F: 5'-GCA TGC TCA CTC TCA AAT CG-3' R: 5'-GGG TTA AGT GGG CTT TG-3'	361 bp	56°C
6. ENSE00003499999	F: 5'-GGA TCT CAA TGT CCC TGC AC-3' R: 5'-TAC CCC AAA ATG ATG GGA CT-3'	330 bp	58°C
7. ENSE00003682693	F: 5'-GCA GGA CAG CAT TGT GAC AG-3' R: 5'-CAG GAC AAA CTG AGA TTA TGG ATG-3'	419 bp	58°C
8. ENSE00001769777	F: 5'-ATG TAA TCT GGC AAA CAA GGG AAG-3' R: 5'-ACA AAG GCA AAG CAG AGA AAG TC-3'	679 bp	59°C

(0 – asymptomatic, 1 – mild, max. 6 events/year, 2 – max. 1 event/month, 3 – more than 12 events/year), and estimation of aC1-INH and aC4 serum level and C1-INH function in plasma (fC1-INH). The fC1-INH was measured by chromotimer (Berichrom-AT, Bering) C1-inhibitor assay (Dade Behring). aC1-INH and aC4 were measured by nephelometry (Dade Behring). The biochemical diagnosis of C1-INH-HAE patients was performed during remission of symptoms and based on evaluation of C1-INH serum level and C1-INH function in plasma [1, 5], which allowed to define the phenotype of C1-INH-HAE as type I and II [13-15].

Genotyping methods: From each patient, the total DNA was extracted from 300 µl of collected blood sample by nucleic acid isolation system QuickGene-Mini80 with DNA blood kit (Kurabo Industries Ltd., Japan) according to manufacturers' protocol. The variation in the *SERPING1* gene coding sequence was detected by Sanger sequencing method (Applied Biosystems 3500 Genetic Analyzer). The *SERPING1* gene coding sequence was checked against the reference sequence No. ENST00000278407.8. Separate analyzes included all 8 exons of the gene. Initially, the PCR amplification was performed with the Hot Start Color Perpetual Taq DNA Polymerase kit (EURx Ltd., Poland). The PCR reaction was carried out in a mixture of 25 µl containing 100 ng DNA, 200 µM dNTP, 1 × Pol Buffer B, 2 × U Hot Start Perpetual Taq DNA polymerase, and 800 nM of primers for each exon. The sequences of the primers are shown in Table 1. For exon 3 and 8, an additional 5% DMSO was used. PCR reaction was performed consisting of denaturation at 95°C for 30 seconds, annealing at primers' dependent temperature (Table 1) for 30 seconds, and extension at 72°C for 45 seconds, 35 cycles. There was an initial 10-minute denaturation at 95°C and a final extension at 72°C for 5 minutes. The PCR products were purified from unincorporated dNTPs and primers prior to the sequencing reaction by Exo-BAP Kit (EURx Ltd., Poland) according to manufacturers' protocol. The final sequencing was performed with BigDye Terminator v3.1 Cycle Sequencing kit (Thermo Fisher Scientific Inc.), and the products were cleaned up with ethanol precipitation and analyzed by Applied Biosystems 3500 Genetic Analyzer. The impact of newly detected sequence variants was predicted *in silico* by MutationTaster [16].

For the patients without point mutations and small deletions/insertions in the *SERPING1* gene sequence, MLPA (Multiple Ligation Dependent Probe Amplification) analysis of large deletion/insertion was performed. SALSA MLPA Probemix P243-B1 SERPING1-F12 (MRC, Holland) was used according to manufacturers' procedure, with a fragment analysis by capillary electrophoresis on the Applied Biosystems 3500 Genetic Analyzers instrument. The data were analyzed by MLPA analysis software (Coffalyser.Net). Statistical analysis of the relationship between the location of detected pathogenic variants and various clinical parameters was carried out by correspondence analysis using Kruskal-Wallis test and χ^2 test depending on the examined parameter.

All individual patients' data were obtained and processed according to patient's consent, approved by the Ethical Committee of the Jagiellonian University in Krakow (KBET 104/B/2014, dated May 22, 2014).

Results

In 28 (68.3%) patients with C1-INH-HAE, the existence of disease-associated variants in the *SERPING1* gene nucleotide sequence (Table 2A) was confirmed using direct Sanger sequencing method, and in 6 patients

Table 2. Disease-associated variants identified by Sanger sequencing genotyping and MLPA analysis in Polish patients with C1-INH-HAE

No. family	No. of case	Sex	Age	C1-INH-HAE type	Disease-associated variant[a]				
A. Sanger sequencing									
					cDNA change	**Protein change**	**Location**	**Mutation type**	**HGMD_MUTATION[b]**
I	1	F	59	I	c.1249+5G>A	?	intron 7	Splice region variant	CS083985
	2	F	30	I	c.1249+5G>A	?	intron 7	Splice region variant	
II	3	F	56	I	c.177_177delC	p.(L60Wfs*19)	exon 3	Deletion variant, frameshift variant	Novel[c] MutationTaster: prediction disease causing, probability – 1
	4	M	36	I	c.177_177delC	p.(L60Wfs*19)	exon 3	Deletion variant, frameshift variant	
	5	F	31	I	c.177_177delC	p.(L60Wfs*19)	exon 3	Deletion variant, frameshift variant	
III	6	M	37	I	c.667C>T	p.(Gln223Ter)	exon 4	Nonsense variant	CM033456
IV	7	F	23	II	c.1396C>T	p.(Arg466Cys)	exon 8	Missense variant	CM890026 associated with type II
	8	F	29	II	c.1396C>T	p.(Arg466Cys)	exon 8	Missense variant	
VI	10	F	69	I	c.1328A>C	p.(His443Pro)	exon 8	Missense variant	CM004572
VIII	15	M	53	II	c.1396C>T	p.(Arg466Cys)	exon 8	Missense variant	CM890026 associated with type II
	16	F	30	II	c.1396C>T	p.(Arg466Cys)	exon 8	Missense variant	
IX	17	F	38	II	c.1397G>T	p.(Arg466Leu)	exon 8	Missense variant	CM92013 associated with type II
X	18	F	22	I	c.463C>G	p.(His155Asp)	exon 3	Missense variant	Novel[c] MutationTaster: prediction disease causing, probability – 0.761[d]
XI	19	F	35	I	c.540_541insAG	p.(Val181Argfs*31)	exon 3	Insertion variant, frameshift variant	Novel[c] MutationTaster: prediction disease causing, probability – 1
XII	20	F	52	I	c.485_498delinsTGCTGAGA	p.(Lys162_ Asn166delinsMetLeuArg	exon 3	Deletion-insertion variant	Novel[c] MutationTaster: prediction disease causing, probability – 0.992
	21	F	55	I	c.485_498delinsTGCTGAGA	p.(Lys162_ Asn166delinsMetLeuArg	exon 3	Deletion-insertion variant	
XVI	25	M	58	I	c.-22-19_-22-4delAGGCTGGCTGGCTCCG	None	intron 1	Splice region variant	Novel[c] MutationTaster: prediction disease causing, probability – 1[d]

Table 2. Cont.

No. family	No. of case	Sex	Age	C1-INH-HAE type	Disease-associated variant[a]				
XIX	28	F	47	II	c.1396C>T	p.(Arg466Cys)	exon 8	Missense variant	CM890026 associated with type II
XX	29	M	44	I	c.467C>A	p.(Ala156Asp)	exon 3	Missense variant	CM087069
XXII	32	F	75	I	c.733dupA	p.(Ser245Lysfs*12)	exon 5	Duplication variant, frameshift variant	Novel[c] MutationTaster: prediction disease causing, probability – 1
XXIII	33	F	51	I	c.990C>G	p.(Tyr330Ter)	exon 6	Nonsense variant	CM960221
XXIV	34	F	41	I	c.622C>T	p.(Gln208Ter)	exon 4	Nonsense variant	Novel[c] MutationTaster: prediction disease causing, probability – 1
XXV	35	M	37	I	c.1322T>C	p.(Met441Thr)	exon 8	Missense variant	CM087089
XXVI	36	F	33	II	c.1396C>A	p.(Arg466Ser)	exon 8	Missense variant	CM900041 associated with type II
XXVIII	38	F	43	I	c.550G>A	p.(Gly184Arg)	exon 3	Missense variant	BM1165483
XXIX	39	M	41	I	c.79dupA	p.(Thr27Asnfs*31)	exon 3	Duplication variant, frameshift variant	Novel[c] MutationTaster: prediction disease causing, probability – 1
XXX	40	F	29	I	c.553_554insG	p.(Ala185Glyfs*72)	exon 4	Insertion variant, frameshift variant	Novel[c] MutationTaster: prediction disease causing, probability – 1
XXXI	41	F	40	I	c.152C>T	p.(Ser51Phe)	exon 3	Missense variant	rs773505671
B. MLPA analysis									
					Change described at genomic DNA	**Protein change**	**Location**	**Mutation type**	
VII	11	F	61	I	rsSERPING1-5(P243-SERPING1 - F12)x3 rsSERPING1-6(P243-SERPING1 - F12)x3	?	exon 5 and 6	Gross duplication	
	12	F	33	I	rsSERPING1-5(P243-SERPING1 - F12)x3 rsSERPING1-6(P243-SERPING1 - F12)x3	?	exon 5 and 6	Gross duplication	

No. family	No. of case	Sex	Age	C1-INH-HAE type	Disease-associated variant[a]			
	13	M	4	I	rsSERPING1-5(P243-SERPING1 - F12)x3 rsSERPING1-6(P243-SERPING1 - F12)x3	?	exon 5 and 6	Gross duplication
	14	F	29	I	rsSERPING1-5(P243-SERPING1 - F12)x3 rsSERPING1-6(P243-SERPING1 - F12)x3	?	exon 5 and 6	Gross duplication
XXI	30	F	58	I	rsSERPING1-8(P243-SERPING1 - F12)x1	?	exon 8	Gross deletions
	31	F	26	I	rsSERPING1-8(P243-SERPING1 - F12)x1	?	exon 8	Gross deletions
Total		26 F 8 M		I – 27 II – 7				

F – female, M – male, [a] all disease-associated variants were present in the heterozygous state, [b] HGMD – the Human Gene Mutation Database, [c] novel disease-associated variant, not previously described, [d] uncertain significance, variant probably leading to C1-INH-HAE, confirmation of functional analysis required

(14.6%) with MLPA method (Table 2B). All together, we found disease-associated variants in exons/introns of *SERPING1* in 34 from 41 individuals (82.9%). In the remaining 7 cases (17.1%), there were no quantitative changes or pathogenic variants in the coding sequence of *SERPING1* gene.

Among 34 patients with confirmed disease-associated variants in the *SERPING1* gene, 27 individuals were diagnosed with C1-INH-HAE type I and 7 with C1-INH-HAE type II. In 31 cases, the course of illness was moderate/severe (score 2-3). In 2 cases, the course of illness was slight (score 1) and in 1 case (a 4-year-old boy with fC1-INH of 65%, carrying gross duplication incl. exon 5-6 in the *SERPING1* gene) was without symptoms of the illness (Table 2B). Main clinical features of analyzed patients with detected disease-associated variants are presented in Supplementary Table 1.

Overall, we identified 20 different disease-associated variants in the *SERPING1* gene sequence by Sanger sequencing analysis, out of which, 13 were found in patients without early familial history of the disease. Among 20 types of detected sequence variants (Table 2A), 11 were previously described and could be found in the HGMD. They were classified pathogenic due to their disruption of splice regions, reporting as nonsense variants as well as missense variants, with well-established impact on C1-INH protein. Furthermore, we detected 9 novel disease-associated variants (not yet described), representing almost half of detected changes, and considered deleterious based on *in silico* predictions. The impact of newly detected sequence variants was predicted by MutationTaster [16]. For all detected variants, the prediction analysis showed their potential disease-causing impact (Table 2A). Furthermore, in some cases (families II, XII), the pathogenic impact of detected variants was confirmed by its family incidence. In cases No. 18 and 25, the variants were considered as variants probably leading to C1-INH-HAE because of the results of *in silico* prediction, but also *de novo* occurrence along with phenotypic symptoms of C1-INH-HAE. Five of detected variants were frameshift variants, detected in exon 3-5 (cases No. 3-5, 19, 32, 39, 40), which in all cases led to translation shifting to another reading frame and establishing premature STOP codons. Moreover, the in-frame deletions/insertions variant was found in exon 3 (cases No. 20 and 21), with the pathogenicity prediction *in silico* and observed by disrupting of the protein function and lowering the activity of C1-INH in patients' blood samples. Single cases of the nonsense variant (case No. 34), missense variant (case No. 18), and deletion in the 5' untranslated regions (UTR) (case No. 25) were also detected. All disease-associated variants were present in the heterozygous state. In all patients with C1-INH-HAE type II, the disease-associated variants described earlier as related to type II (missense point mutation in Arg466, equivalent to the position Arg444) were found [17, 18].

Table 3. Clinical characteristic 7 of 41 (17.1%) patients without confirmation of disease-associated variants by Sanger sequencing genotyping and MLPA analysis

No. family	No. of case	Sex	Age	aC1-INH (g/l)	fC1-INH (%)	aC4 (g/l)	C1-INH-HAE type	Family history (yes/no)	Score (0-3)	Age of onset/ location of symptoms	Detected polymorphism
V	9	F	23	0.06	19.4	0.04	I	Yes	3	4/abdomen	exon 8: rs4926. c.1438G>A (p.Val480Met) in heterozygous state
XIII	22	M	67	0.08	47	0.12	I	No	1	20/foot	exon 8: rs4926. c.1438G>A (p.Val480Met) in heterozygous state
XIV	23	F	49	0.048	13.6	0.11	I	Yes	2	15/abdomen	exon 8: rs4926. c.1438G>A (p.Val480Met) in heterozygous state
XV	24	F	53	0.048	23	0.07	I	No	2	5/abdomen	exon 8: rs4926. c.1438G>A (p.Val480Met) in heterozygous state
XII	26	F	47	0.1	46	0.07	I	Yes	2	24/abdomen	exon 8: rs4926. c.1438G>A (p.Val480Met) in heterozygous state
XVIII	27	F	34	0.02	30.4	0.03	I	Yes	3	10/abdomen	exon 8: rs4926. c.1438G>A (p.Val480Met) in heterozygous state
XXVII	37	M	75	0.05	10	0.03	I	No	2	62/face	exon 8: rs4926. c.1438G>A (p.Val480Met) in heterozygous state
Total		6 F 2 M		0.6	27.3	0.1		Yes – 4 No – 3	0-3 (median, 2.0)	4-62 (mean, 21.1)	

aC1-INH – serum level of C1-INH (normal range, 0.2-0.39 g/l), fC1-INH – activity of C1-INH in plasma (normal range, 70-130%), aC4 – serum level of C4 (normal range, 0.1-0.4 g/l)

Gross duplication/deletion was identified by MLPA analysis in 6 patients (5 females and 1 boy) in two families (Table 2B) and all suffered from C1-INH-HAE type I. Four patients had a positive family history, with moderate/severe course of the disease. One patient (4-year-old boy) was asymptomatic. The age of onset in the next 5 patients ranged between 10 and 22 years old (mean, 13.8 years old). In 2 cases, the location of first symptom concerned the abdomen, the face was in 2 cases, and larynx in 1 patient. In 6 individuals with identified mutation in MLPA method, we found duplication incl. exon 5 and 6 in 4 cases from family nr VII and deletion incl. exon 8 in 2 individuals from family XXI. Quantitative changes described at genomic DNA level detected by MLPA are noted in the Human Gene Mutation Database as correlated with C1-INH-HAE.

The analysis of 7 (17.1%) remaining patients (5 females and 2 males; mean age, 49.7 years old) with C1-INH-HAE without mutations in Sanger sequencing and MLPA genotyping is presented in Table 3. All patients were recognized as suffering from C1-INH-HAE type I, with moderate and severe course of illness. The age of onset of a disease ranged from 4 to 62 years old (mean, 21.1 years old). In 5 cases, the first symptoms of disease were an abdominal attack. Four individuals had a positive family history. All these patients had the same type of polymorphism in exon 8: c.1438G>A (p.Val480Met).

We did not find significant relations between the location of disease-associated variants in the *SERPING1* gene and aC1-INH, fC1-INH, or aC4 parameters. Moreover, the analysis of 17 patients with a severe course of illness did not reveal any correlations with the type or location of disease-associated variants. The early onset of symptoms (< 10 yrs.) was noted in 34.1% of patients. Till the age of 18, 80.5% of patients were symptomatic. In patients with an early age of onset of C1-INH-HAE, type I was dominating. There was no correlation between the location of disease-associated variants and the age of manifestation of the disease symptoms, family history, and the location of first edema. Most of the patients (74%) revealed symptoms of illness below 14 yrs. The abdomen was the most frequent location of first symptoms of C1-INH-HAE < 18 yrs.

The most often disease-associated variations in the *SERPING1* gene was located in exon 8. Among 13 patients with a disease-associated variant in this region of the *SERPING1* gene, we observed 7 patients with C1-INH-HAE type II and 6 patients with C1-INH-HAE type I. In all patients with type II, missense point mutation in

Arg466 was found (Table 2A). In these patients, aC1-INH was normal or elevated, and ranged from 0.2 to 0.7 g/L (mean, 0.33 g/L), and the value of fC1-INH was low and ranged from 11.8 to 37.1% (mean, 23.3%). In remaining 6 patients with type I, various disease-associated variants were located in whole sequence of exon 8 (Table 2A). In 2 cases, splice region variants were found, and gross deletions incl. exon 8 were detected in another 2 cases. The aC1-INH in these patients was very low and ranged from 0.04 to 0.09 g/L (mean, 0.06 g/L). Furthermore, the value of fC1-INH was very low and ranged from 0 to 22.4% (mean, 14.25%). The onset of C1-INH-HAE as well the course of illness did not differentiate the patients with disease-associated variant in exon 8 suffer with type II and I of C1-INH-HAE.

Statistical analysis revealed significant relationship only between the location of mutation in exon 8 and type II C1-INH-HAE ($p = 0.01017$). We did not find any relation between the type and location of disease-associated variants and the course of disease, location of symptoms, age of onset, family history, and the activity of C1-INH.

Discussion

C1-INH-HAE [1, 5, 9] is a rare and life-threatening disease, with an autosomal dominant trait. Currently, genotyping is very important diagnostic tool in patients suspected of C1-INH–HAE, especially in cases with no family history of the disease or relatives of patients with C1-INH-HAE, without evident symptoms of C1-INH-HAE [2, 3]. The genetic research of this disease allows to search for new strategies of therapies [4, 11] as well as possible *ex vivo* gene correction in future [11, 19]. Over 500 mutations have been found until now in the *SERPING1* gene, which are associated with C1-INH-HAE, according to the Human Genome Mutation Database [4].

The presented results are the first genetic studies in Polish patients with C1-INH-HAE type I and II [12]. Using classic genotyping by Sanger sequencing and quantitative MLPA analysis, we observed the presence of disease-associated variants in 34 out of 41 patients (82.9%), according to previous data concerning other European populations [7, 10, 20-23].

In the remaining 7 patients (17.1%), no disease-associated variants in the *SERPING1* gene were found. In all these patients, polymorphism: c.1438G>A (p.Val480Met) in exon 8 was present (Table 3). However, the frequency of this polymorphism is quite high in the general population (according to Ensembl database, the frequency of variant c.1438A is 27%, rs4926). Until now, this polymorphism has not been related to C1-INH-HAE (ClinVar: RCV000288563.1, RCV000616583.1 – clinical significance: benign), but there may be preliminary observation indicating the predisposition of people with this variant to C1-INH-HAE type I unrelated to the presence of disease-associated variants in encoding sequence of the *SERPING1* gene. All other clinical and laboratory parameters, except for significantly higher mean age of onset of C1-INH-HAE, did not differentiate this group in comparison with patients with disease-associated variants.

It seems that C1-INH-HAE is a genetically complex disorder [1, 4], with participation genetic changes also in other than the *SERPING1* gene, as genes encoding F12, aminopeptidases, angiopoetin1, plasminogen, or bradykinin B2/B1 receptors genes, which also may modify the phenotype of C1-INH-HAE [1, 4, 24-29]. It is possible that more precise, modern methods of genome analysis, such as Next-Generation Sequencing (NGS) [2, 30-32], may disclose additional genetic changes and allow to increase the percentage of patients with disease-associated variants in C1-INH-HAE. Such possible confirmed NGS analysis was performed in another study [32] and in 7 our patients with negative genetic analysis, identified one case (female aged 47 with type I C1-INH-HAE), another likely pathogenic missense variant in exon 4 of the *SERPING1* (c.629T> C, p.(Leu210Pro) in heterozygous).

Our results confirm the presence of large diversity of disease-associated variants in the *SERPING1* gene located in various regions for all 8 exons as well introns of the *SERPING1* gene in patients with C1-INH-HAE. Disease-associated variants in the *SERPING1* gene were located mainly in exon 3 (36.4%) and in exon 8 (22.7%). The most frequent were missense variants (40.9%) and deletion/insertion variants (18.2%), nonsense variants (13.6%), duplication (9.1%), gross duplication/deletions (9.1%), and splice region/regulatory variants (9.1%) (Table 2). These findings agree with previous data reported by other authors [2, 6, 10, 33, 34]. The cases of patients with C1-INH-HAE with homozygous C1-INH deficiency were also noted by other authors [35], which was not observed in our study.

The location and type of disease-associated variants in patients of the same 6 family included into study were identical, which confirms previous observations of other authors [34], and what seems to be connected with the dominant type of inheritance with high penetrance. We confirmed that despite the same type and location of a disease-associated variant, the course of illness and the age of onset of C1-INH-HAE among the members of the same family was diverse, what indicates that the clinical phenotype does not directly correlate with the genotype, and the phenomenon of variable expressivity can be observed [2, 36]. It may suggest various personal (metabolic/environmental) conditions having an essential influence on the course of disease in patients with C1-INH-HAE, which was emphasized by Ameratunga *et al.* [11], independently on genotype [1, 2, 8, 9, 26, 29].

Independently of the type of disease-associated variants in the *SERPING1* gene in patients with C1-INH-HAE type I, low fC1-INH value and low aC1-INH serum level are characteristic diagnostic biomarkers. However in all 7 patients with C1-INH-HAE type II, characteristic low values of fC1-INH and normal values of aC1-INH were

strictly connected with disease-associated variants described earlier [17, 18], as associated with type II (missense point mutation in Arg466) located in exon 8 in the critical hinge region and reactive center of the C1-INH molecule [37].

Studies performed during remission suggest that neither the constant presence in patients of disease-associated variants in the *SERPING1* gene nor low values of aC1-INH as well as low values of fC1-INH are the conditions *sine qua non* of the presence of symptoms of C1-INH-HAE in patients [4, 11, 38]. However, it seems that only fC1-INH value is more associated with the activity of C1-INH-HAE symptoms, as suggested by others authors [4, 38] and confirmed in our study in only one case of asymptomatic 4-years-old boy, with identified gross duplication incl. exon 5 and 6 and with close to normal value of fC1-INH of 65%. It seems that an impact of additional, triggering factors that influence disease-severity are only essential for the provocation of angioedema attack in patients with C1-INH-HAE.

Our findings confirm previous reports [1, 5, 8, 9, 11] that most patients with C1-INH-HAE reveal first symptoms of illness before the age of 14, and that C1-INH-HAE type I as well as positive family history dominates in this group of patients. According to some authors [8, 39], the early onset of C1-INH-HAE often predisposes to a severe course of illness, but in our patients with severe course of disease, the early onset (< 10 years) was noted only in 50% of cases with type I and type II C1-INH-HAE.

A very interesting group constitutes the patients with disease-associated variants in the *SERPING1* gene located in exon 8, focusing the cases with type I and type II C1-INH-HAE, for which the mechanisms regulating functional activity and antigenic level of C1-INH are different [4]. In all these cases, the value of fC1-INH was very low. However, aC1-INH antigenic level was low in type I, and normal or somewhat elevated in type II. The disease-associated variants located in exon 8 in type I significantly varied and were in different region of exon 8. In comparison, the disease-associated variants in type II were located in all 7 cases in one region of exon 8, close to the region encoding the reactive center loop (RCL) of the C1 molecule responsible for functional activity of C1-INH. All are missense point mutations, concerning codon for Arg466. Rarely, other mutations can cause C1-INH-HAE type II, like in the amino acid residue Lys251, which affect only the function of C1-INH [40]. It became generally accepted that mechanisms regulating C1-INH activity as well as responsibility for C1 antigenic level in type I and II C1-INH-HAE are different [4]. In C1-INH-HAE type I, the disease-associated variants in the *SERPING1* gene affect the synthesis and secretion of C1-INH. However, in C1-INH-HAE type II, molecules of mutant C1-INH are secreted but dysfunctional, because the mutation is located in the *SERPING1* gene region essential for activity of C1-INH. This type of mutations may protect patients with type II from the decrease of aC1-INH level by influencing mRNA transcription or secretion of C1-INH in the serum, or may decrease the catabolism of aC1-INH elevated usually in patients with C1-INH-HAE [35, 41].

Better understanding of the pathomechanism leading to the development of these two different types of C1-INH-HAE [1, 4, 11, 38] may help to explain the differences in values of aC1-INH serum level and fC1-INH caused by diverse genetic changes. At last, it may also help to understand various cases, such as those described by Jasenack *et al.* [42], who presented 7 individuals from one family with C1-INH HAE type I with an unusual novel mutation of *SERPING1* gene in exon 8, in the region typical for a mutation associated with type II.

It is generally known and accepted that in patients with C1-INH-HAE despite the same mutation, the course of disease is different [1, 2, 4, 8, 9, 43] and the frequency and location of symptoms of the disease undergo changes in the same patient. Furthermore, the history in some patients indicates that the period of severe symptoms is followed by a long remission without an evident reason and without the influence on aC1-INH and fC1-INH values. The question is why some people, despite very low values of aC1-INH and fC1-INH, are asymptomatic or live for a long time without symptoms. All these facts seem to indicate that C1-INH-HAE is a disorder with very composed pathomechanism, whose symptoms are related not only to genetic changes but also to additional unspecific factors disturbing homeostasis of the human body [4, 11, 38].

Acknowledgements

This study was supported by a grant from the National Center of Science No. UMO-2014/13/B/NZ6/00246.

The preliminary results (in the form of poster) were presented on 9th and 10th C1-inhibitor Deficiency Workshop, Budapest 2015 and 2017.

The authors declare no conflict of interest.

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